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Preliminary

cDNA Cloning of Isocitrate Lyase from the Copper-Tolerant Wood-Rotting Basidiomycete *Fomitopsis palustris*[*1]

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Introduction

Wood-rotting basidiomycete *Fomitopsis palustris* accumulates oxalic acid in the culture fluid in 80% theoritical yield on the basis of glucose consumed[1]. Oxalate produced by wood-rotting fungi, including *F. palustris*, has been proposed to play important biochemical roles in wood decay processes[2]. In addition, oxalate inactivates copper contained in wood preservatives by forming oxalate-copper complex[3].

Quite recently, *F. palustris* has been proposed to acquire energy by oxidizing glucose to oxalate[1]. In this unprecedented metabolic system, isocitrate lyase (ICL), which is shared by the TCA and glyoxylate cycles, has been revealed to be a pivotal enzyme involved in a new biochemical device for producing oxalate in *F. palustris*. Interestingly, ICL is produced as a constitutive enzyme, because the enzyme synthesis was not suppressed by glucose[4]. These characteristic features of the enzyme are in sharp contrast to those of ICLs from many other microorganisms, including *Escherichia coli*, *Saccharomyces cerevisiae*, and *Aspergillus nidulans*. Thus, to elucidate the regulatory mechanism for the gene expression of ICL in *F. palustris*, cDNA cloning for the ICL was attempted, because no molecular investigation of *F. palustris* ICL has been conducted. A cDNA fragment encoding 532 amino acids, which represents almost whole part of the *F. palustris* ICL, has been cloned.

Materials and Method

λZAPII cDNA library was constructed by use of poly(A)$^+$ RNA prepared from *F. palustris* (IFO 6137) cultivated as previously reported[4,5]. RT-PCR was performed by using primers designed on the basis of consensus amino acid sequences of *Aspergillus nidulans* ICL[6]. The purified ICL of *F. palustris*[7] was digested with Lysylendopeptidase. The amino acid sequences of the polypeptides thus obtained were determined by Applied Biosystems 491 Protein Sequencer.

Results and Discussion

RT-PCR was performed to obtain a nucleotide fragment encoding the cDNA of *F. palustris* ICL. The resulting PCR fragment (770 bp) contained sequences (KLEQFLRRVAHLSYP, KEFADGV and KAFV) determined for internal amino acids of the purified *F. palustris* ICL[7] and other consensus amino acid sequences reported for ICLs[6,8]. Thus, the cDNA library was screened by plaque hybridization with the obtained PCR fragment labelled with [α-^{32}P] dATP. Several positive clones were obtained. A cDNA insert (1714 bp) was predicted to contain an open reading frame (532 amino acids), although the cDNA fragment lacked 5′ end. The fragment contained another two internal amino acid sequences (KAYVELVQRRE and KGAA) of *F. palustris* ICL besides the three described above. Therefore the results show that the cDNA fragment thus obtained encodes most part of *F. palustris* ICL. Moreover, the deduced amino acid sequence of this fungus shows 77% identity to that of the basidiomycete *Coprinus cinereus* ICL[8].

The previous study with purified *F. palustris* ICL[7] reported that a thiol group of cysteine residue might be involved in the catalytic function. The important functions of the thiol group for ICLs of *Neurospora crassa*[9,10] and *E. coli*[11] have also been reported. The present study shows that the cysteine residue is located in the conserved region (KKCGHM) in the obtained cDNA fragment and this cysteine residue is strongly suggested to play an important role in the catalytic function of *F. palustris* ICL.

ICL of *F. palustris* requires Mg^{2+} for catalytic activity like ICLs of other creatures. In the case of *A. nidulans* ICL, D114, D168, D170, and E197 have been proposed to form a cluster for the Mg^{2+}-binding site[12]. The present study shows that the same amino acids are conserved in the cDNA fragment. Thus, the cluster of four carboxyl groups of these amino acids is strongly suggested to be important for Mg^{2+}-binding of *F. palustris* ICL.

The deduced amino acid sequence of the cloned cDNA fragment shows high identity and similarity to those of *C. cinereus*[8] and *A. nidulans*[6]. However, the sequence, KLEQFLRRVAHLSYP described above, has no identity to any ICL of eukaryotes, including *C. cinereus* and *A. nidulans*. Furthermore, the sequence was found in the region, which has been proposed to play a role in inclusion of the eukaryotic ICLs in peroxisomal microbodies.

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Thus, the elucidation of a role of the sequence may be important for locating the enzyme in subcellular sites. Furthermore, *F. palustris* ICL has been reported to be a trimeric enzyme[7], which is contrasted with the findings that most reported ICLs are tetrameric enzymes. Thus, the cloning of the complete cDNA of *F. palustris* ICL is needed to elucidate why *F. palustris* ICL is a trimeric enzyme. The cloned cDNA fragment obtained in this study would be helpful for not only achievement of complete cDNA cloning but also genomic DNA cloning of ICL for the elucidation of the expression regulatory mechanism of *F. palustris* ICL.

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